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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/823,784	04/14/2004	Karen Uhlmann	3035-101	4952

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EXAMINER	
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1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/823,784	UHLMANN ET AL.	
	Examiner	Art Unit	
	Amanda M. Shaw	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 May 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-5,7-20 and 22-38* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-5,7-20 and 22-38* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *8/17/2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/14/2007 has been entered.

Claims 1-5, 7-20, and 22-38 are currently pending. Claims 1, 7, 10, 12, 17, 18, 22, 25, 32, and 34 have been amended. Claims 35-38 are newly presented. Therefore Claims 1-5, 7-20, and 22-38 will be addressed herein.

Withdrawn Rejections

2. The rejection made under 35 USC 112 2nd paragraph in section 2 of the previous office action is withdrawn in view of arguments.

The rejections made under 35 USC 103(a) in sections 4-6 of the previous office action is withdrawn in view of arguments.

Information Disclosure Statement

3. The listing of references in the specification (See pages 28-30) is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP

§ 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Specification

4. Applicant is reminded of the proper language and format for an abstract of the disclosure.

The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. It is important that the abstract not exceed 150 words in length since the space provided for the abstract on the computer tape used by the printer is limited. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details.

The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc.

The abstract of the disclosure is objected to because the abstract appears to exceed 150 words and the abstract contains legal phraseology (i.e., "said nucleic acid molecule"; "said nucleotide"; "said agent" etc.) Correction is required. See MPEP § 608.01(b).

The specification is also objected to because pages 21-23 contain multiple sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). Where the specification shows a sequence that is set forth in the "Sequence Listing" reference

must be made to the sequence by use of the sequence identifier, preceded by "SEQ ID NO:" in the text of the specification.

Claim Objections

5. Claim 22 is objected to because this claim is a duplicate of claim 7. Appropriate correction is required.

Claim Rejections - 35 USC § 112

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 1-5, 7-20, and 22-38 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-5, 7-20, and 22-31, and 33-38 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. Step (b) of claims 1 and 12 recites "amplifying said nucleic acid molecule treated with said agent via at least one amplification primer" and step (c) of claims 1 and 12 recites "real time sequencing said single stranded amplified nucleic acid". These steps are considered unclear because typically when a nucleic acid is amplified with a primer the amplification product is a double stranded nucleic acid. Therefore it seems like there is method step missing, i.e.,

a step of making the double stranded amplification product single stranded. Further the claim states that the amplification primer has a label that "forms an anchor for removal of single stranded amplified nucleic acid molecules". It is unclear if the anchor is just a property of the label or if the anchor is actually used to remove single stranded amplified nucleic acid molecules so that they can be utilized in step (c).

Claims 8, 23, and 37 recite the limitation "said methylated nucleotide". There is insufficient antecedent basis for this limitation in the claims because although previous claims refer to detecting whether said nucleotide is methylated or not methylated, the previous claims do not refer to a "methylated nucleotide". It is suggested the claims be amended to overcome this rejection by e.g., replacing "said" with "a".

Claims 9 and 24 are indefinite over the recitation of steps (b) and (c). Specifically step (b) recites, "addition of a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine-phosphosulfate (APS) and luciferin" and step (c) recites, "sequential addition of all four different dNTPs". These steps are considered indefinite because it is unclear if these reagents are being added to a mixture containing the sequencing primer and amplified nucleic acid molecule in single stranded form or if they are being added to something else.

Claims 9 and 24 are indefinite over the recitation of steps (c) and (d). Specifically step (c) recites, "sequential addition of all four different dNTPs" and step (d) recites, "detection of a luminescent signal...". These steps are considered indefinite because it is unclear if one can detect the luminescent signal only after all four dNTPs have been

added sequentially or if one should check for a luminescent signal after the addition of the first, second, third, and forth dNTP.

Claims 9 and 24 are indefinite over the recitation of the phrase "four different dNTPs". This phrase is considered indefinite because it is unclear which four dNTPs are being used.

Claims 9 and 24 are indefinite over the recitation of "wherein an intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide." Because the term "correlated" has not been clearly defined in the specification and there is no art recognized definition for this term the relationship between the luminescent signal and a nucleotide being incorporated is unclear. For example it is unclear if a nucleotide is incorporated when there is a high intensity signal or low intensity signal. Further the claim is indefinite over the recitation of the phrase "wherein the intensity of said signal is indicative of the methylation status of said nucleotide". This phrase is confusing because it is unclear how the intensity of the signal is related to the methylation status of the nucleotide. For example it is unclear if a methylated nucleotide would give off a high intensity signal or low intensity signal.

Claims 12-20, 23-26, 28-29, and 38 are indefinite because the claims do not clearly set forth a step of diagnosing a pathological condition or providing a prognosis for a pathological condition. The claims do not recite a clear nexus between the preamble and the last step of the method because detecting whether a nucleotide is methylated or not methylated is not equivalent to diagnosing a pathological condition or providing a prognosis for a pathological condition. Additionally the claim does not

clearly indicate the relationship between the methylated nucleotide and any pathological condition. Thus it is unclear how detecting a methylated nucleotide sequences allows for the diagnosis and/or prognosis of a pathological condition.

Claim 32 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. Step (c) of claim 32 recites "amplifying said nucleic acid molecule via at least one amplification primer..." and step (d) of claim 32 recites "real time sequencing said single stranded amplified nucleic acid". These steps are considered unclear because typically when a nucleic acid is amplified with a primer the amplification product is a double stranded nucleic acid. Therefore it seems like there is method step missing, i.e., a step of making the double stranded amplification product single stranded. Further the claim states that the amplification primer has a label that "forms an anchor for removal of single stranded amplified nucleic acid molecules". It is unclear if the anchor is just a property of the label or if the anchor is actually used to remove single stranded amplified nucleic acid molecules so that they can be utilized in step (d).

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and

the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 1-5, 7-9, 11-12, 19-20, 22-24, 26-33, and 36-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al (Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568 Issued 2001).

Regarding Claims 1-5, 7-9, 11, 22, 27, 30-31, 33, and 36-37 Uhlmann et al teach a method for identifying methylated cytosines comprising treating a sample containing genomic DNA derived from blood and tumor tissue with sodium bisulfite and amplifying the sample by PCR. Uhlmann et al further teach that the amplified nucleic acids were sequenced by the dideoxynucleotide chain termination method determine the methylation state of the amplified product (Page 1750-1751).

Uhlmann et al do not teach a method wherein the amplification primer has a label that forms an anchor for removal of single stranded amplified nucleic acid molecules.

Further Uhlmann does not teach that the amplified nucleic acids were sequences using a real-time sequencing method.

However, Nyren et al teach a real time sequencing method called pyrosequencing that can be used to identify a base at a predetermined position in a DNA sample using an extension primer, which hybridizes immediately adjacent to the target position. The DNA sample and extension primer are subjected to a polymerase reaction in the presence of each dNTP. The dNTPs are successively added to the same sample primer mixture and the dNTPs will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position. When the PPi is released a certain amount of light gets released that is equivalent to the amount of incorporated nucleotides. The unincorporated dNTPs get degraded (Column 2, lines 25-42). The method of Nyren can be used to determine base changes caused by methylation in samples treated with sodium bisulfite because the treatment converts unmethylated cytosine to uracil which gets amplified as thymine. Methylated cytosine remains unchanged and gets amplified as guanine. Therefore if an adenine gets incorporated then one would know that they cytosine was methylated. If a cytosine gets incorporated one would know that the cytosine was unmethylated. Nyren further teach to aid in the separation of a single stranded sample DNA from its complementary strand the sample DNA may be provided with means for attachment to a solid support. Nyren teaches that one or more of the PCR primers may carry a functional group such as a biotin which permits subsequent immobilization (Column 8, lines 22-31).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann et al by using pyrosequencing to determine the sequence of the amplified DNA fragment as suggested by Nyren. Specifically Nyren et al teaches that the benefit of performing pyrosequencing over other sequencing methods such as the enzymatic chain termination method of Sanger is that pyrosequencing enables a base to be identified in a target position and DNA to be sequenced simply and rapidly while avoiding the need for electrophoresis and use of harmful radiolabels (Column 1, lines 60-64). Nyren further teach that other sequencing methods which rely on electrophoresis are not well suited for large-scale genome projects or clinical sequencing where high throughput is needed (Column 1, lines 15-30). However the pyrosequencing method of Nyren opens up the possibility for an automated approach for large scale, non-electrophoretic sequencing procedures which allow for continuous measurement of the progress of the polymerization reaction with time. The method of Nyren also has the advantage that multiple samples may be handled in parallel (Column 9, lines 4-6).

Regarding Claim 12 as noted in the MPEP 211.02, " a preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone." Further, in *Pitney Bowes Inc. v. Hewlett-Packard Co.*, 182F.3d 1298, 1305, 51 USPQ2d 1161, 1166 (Fed Cir. 1999) the court held that if the body of

the claim sets forth the complete invention, and the preamble is not necessary to give "life, meaning and vitality" to the claim, "then the preamble is of no significance to claim construction because it cannot be said to constitute or explain a claim limitation." In the present situation, the steps present in the method are able to stand-alone and the preamble limitation is not accorded patentable weight. Accordingly, the claim language of "a method for the diagnosis of a pathological condition or the predisposition for a pathological condition" merely sets forth the intended use or purpose of the claimed method, but does not limit the scope of the claims.

Regarding Claims 12, 19-20, 23-24, 26, 28-29, Uhlmann et al teach a method comprising treating a sample containing genomic DNA derived from blood and tumor tissue with sodium bisulfite and amplifying the sample by PCR. Uhlmann et al further teach that the amplified nucleic acids were sequenced by the dideoxynucleotide chain termination method determine the methylation state of the amplified product (Page 1750-1751).

Uhlmann et al do not teach a method wherein the amplification primer has a label that forms an anchor for removal of single stranded amplified nucleic acid molecules. Further Uhlmann does not teach that the amplified nucleic acids were sequences using a real-time sequencing method.

However, Nyren et al teach a real time sequencing method called pyrosequencing that can be used to identify a base at a predetermined position in a DNA sample using an extension primer, which hybridizes immediately adjacent to the target position. The DNA sample and extension primer are subjected to a polymerase

reaction in the presence of each dNTP separately and the dNTPs will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position. When the PPi is released a certain amount of light gets released that is equivalent to the amount of incorporated nucleotides. The unincorporated dNTPs get degraded (Column 2, lines 25-42). The method of Nyren can be used to determine base changes caused by methylation in samples treated with sodium bisulfite because the treatment converts unmethylated cytosine to uracil which gets amplified as thymine. Methylated cytosine remains unchanged and gets amplified as guanine. Therefore if an adenine gets incorporated then we would know that they cytosine was methylated. If a cytosine gets incorporated we would know that the cytosine was unmethylated. Nyren further teach to aid in the separation of a single stranded sample DNA from its complementary strand the sample DNA may be provided with means for attachment to a solid support. Nyren teaches that one or more of the PCR primers may carry a functional group such as a biotin which permits subsequent immobilization (Column 8, lines 1-31).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann et al by using pyrosequencing to determine the sequence of the amplified DNA fragment as suggested by Nyren. Specifically Nyren et al teaches that the benefit of performing pyrosequencing over other sequencing methods such as the enzymatic chain termination method of Sanger is that pyrosequencing enables a base to be identified in a target position and DNA to be sequenced simply and rapidly while avoiding the need

for electrophoresis and use of harmful radiolabels (Column 1, lines 60-64). Nyren further teach that other sequencing methods which rely on electrophoresis are not well suited for large-scale genome projects or clinical sequencing where high throughput is needed (Column 1 lines 15-36). However the pyrosequencing method of Nyren opens up the possibility for an automated approach for large scale, non-electrophoretic sequencing procedures which allow for continuous measurement of the progress of the polymerization reaction with time. The method of Nyren also has the advantage that multiple samples may be handled in parallel (Column 9, lines 4-6).

Regarding Claim 32 as noted in the MPEP 211.02, the courts have stated that " a preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone." In the present situation, the steps present in the method are able to stand-alone and the preamble limitation is not accorded patentable weight. Accordingly, the claim language of "a method for generating new nucleotide pairing partners upon amplification of at least one nucleic acid molecule for the detection of the methylation status of nucleotides of said nucleic acid molecule" merely sets forth the intended use or purpose of the claimed method, but does not limit the scope of the claims.

Regarding Claim 32, Uhlmann et al teach a method comprising treating a sample containing genomic DNA derived from blood and tumor tissue with sodium bisulfite and

amplifying the sample by PCR. Uhlmann et al further teach that the amplified nucleic acids were sequenced by the dideoxynucleotide chain termination method determine the methylation state of the amplified product (Page 1750-1751).

Uhlmann et al do not teach a method wherein the amplification primer has a label that forms an anchor for removal of single stranded amplified nucleic acid molecules. Further Uhlmann does not teach that the amplified nucleic acids were sequences using a real-time sequencing method.

However, Nyren et al teach a real time sequencing method called pyrosequencing that can be used to identify a base at a predetermined position in a DNA sample using an extension primer, which hybridizes immediately adjacent to the target position. The DNA sample and extension primer are subjected to a polymerase reaction in the presence of each dNTP separately and the dNTPs will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position. When the PPi is released a certain amount of light gets released that is equivalent to the amount of incorporated nucleotides. The unincorporated dNTPs get degraded (Column 2, lines 25-42). The method of Nyren can be used to determine base changes caused by methylation in samples treated with sodium bisulfite because the treatment converts unmethylated cytosine to uracil which gets amplified as thymine. Methylated cytosine remains unchanged and gets amplified as guanine. Therefore if an adenine gets incorporated then we would know that they cytosine was methylated. If a cytosine gets incorporated we would know that the cytosine was unmethylated. Nyren further teach to aid in the separation of a single stranded sample DNA from its

complementary strand the sample DNA may be provided with means for attachment to a solid support. Nyren teaches that one or more of the PCR primers may carry a functional group such as a biotin which permits subsequent immobilization (Column 8, lines 1-31) .

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann et al by using pyrosequencing to determine the sequence of the amplified DNA fragment as suggested by Nyren. Specifically Nyren et al teaches that the benefit of performing pyrosequencing over other sequencing methods such as the enzymatic chain termination method of Sanger is that pyrosequencing enables a base to be identified in a target position and DNA to be sequenced simply and rapidly while avoiding the need for electrophoresis and use of harmful radiolabels (Column 1 lines 60-64). Nyren further teach that electrophoresis is not well suited for large-scale genome projects or clinical sequencing where high throughput is needed (Column 1, lines 15-36). However the pyrosequencing method of Nyren opens up the possibility for an automated approach for large scale, non-electrophoretic sequencing procedures which allow for continuous measurement of the progress of the polymerization reaction with time. The method of Nyren also has the advantage that multiple samples may be handled in parallel (Column 9, lines 4-6).

9. Claims 13-16, 18 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al (Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568 Issued 2001) as applied to claim 12 above, and in further view of Herman (U.S. Patent 5786146 Issued 1998).

The teachings of Uhlmann et al and Nyren et al are presented above.

The combined references do not teach that the methylation status is used to diagnose a pathological condition such as cancer, a neurodegenerative disease or another neurological disorder. The combined references also do not teach that the methylation status is used diagnose cancer that is a primary tumor, a metastasis or a residual tumor. The combined references do not teach that the primary tumor is a glioma selected from the group comprising: astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, and a pilocytic astrocytoma. The combined references also do not teach that the neurological disorder is selected from the group comprising: Prader-Willi-Syndrome, Angelman-Syndrome, Fragile-X-Syndrome, or ATR-X-Syndrome.

However, Herman et al teaches that the detection of methylated CpG containing nucleic acid is indicative of several disorders. Such disorders include but are not limited to low grade astrocytoma, anaplastic astrocytoma, glioblastoma, medulloblastoma, colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, endometrial cancer and neuroblastoma. Identification of methylated CpG status is also

useful for detection and diagnosis of genomic imprinting, fragile X syndrome and X-chromosome inactivation (Column 10, lines 49-58).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method of Uhlmann and Nyren to diagnose pathological disorders. It was well known in the art at the time the invention was made that the detection of methylated sequences is indicative of several pathological disorders. Accordingly, one of ordinary skill in the art would have been motivated to use the method of Uhlmann and Nyren in order to have achieved the advantage of being able to diagnose these diseases.

10. Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) and Herman (U.S. Patent 5786146 Issued 1998) as applied to claims 12 and 38 above, and in further view of Feinberg (Pub No. US 2003/0232351).

The teachings of Uhlmann, Nyren, and Herman are presented above.

The combined references do not teach a method used to diagnose neurodegenerative diseases such as Alzheimer's disease, Parkinson disease, Huntington disease, or Rett-Syndrome.

However, Feinberg teaches a method of determining a disease state in a subject by determining DNA methylation status. Although the disease state is often cancer, the methods taught by Feinberg also include Alzheimer's disease and Parkinson's disease (Paragraph 0029).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method that Uhlmann, Nyren and Herman used to diagnose primary tumors, to also diagnose neurodegenerative diseases. It was well known in the art at the time the invention was made that the detection of methylated sequences is indicative of certain neurodegenerative diseases. Accordingly, one of ordinary skill in the art would have been motivated to use the method of Uhlmann, Nyren and Herman in order to have achieved the advantage of being able to diagnose these diseases.

11. Claims 10, 25, and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claims 1 and 12 above, and in further view of Sylvan (US Patent 7078168 Filed 2/2002).

The teachings of Uhlmann and Nyren are presented above.

The combined references do not teach a method further comprising calculating a frequency of methylated nucleotides from the results of said real time sequencing. Further the combined references do not teach a method wherein an allele frequency of 5% can be detected.

However, Sylvan teaches a method of determining the frequency of an allele in a population of nucleic acid molecules. The method comprises performing primer extension reactions using a primer which binds at a predetermined site located in nucleic acid molecules and obtaining a pattern of nucleotide incorporation (Abstract).

Specifically Fig 4a depicts graphically relative peak heights from a pyrosequencing reaction plotted against allele frequency. After the pyrosequencing was performed, the resulting peak heights were plotted versus expected allele frequency and a linear relationship between the 2 was demonstrated (Column 5, lines 7-16). As you can see an allele frequency of 5% can be detected.

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method that Uhlmann and Nyren by further calculating the frequency of methylated nucleotides from the results of the pyrosequencing as suggested by Sylvan. The method of Sylvan is advantageous in that it determines the exact sequence of a nucleic acid fragment while directly measuring the amount of nucleotide incorporated. Using this method is it possible to obtain accurate, cost effective, and rapid information on allele frequencies (Column 22, lines 39-67 and Column 23, lines 1-4).

12. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claim 1 above, and in further view of Laird (US 2002/0086324 Filed 10/2001).

The teachings of Uhlmann and Nyren are presented above.

The combined references do not teach a method wherein the amplification primer does not comprise CpG.

However, Laird teaches a method wherein a genomic DNA is provided that has mixed methylation status. The sample is converted in a standard sodium bisulfite

reaction and the mixed products are amplified by a PCR reaction using primers that do not overlap any CpG dinucleotides. This produces an unbiased (with respect to methylation status) heterogeneous pool of PCR products. The mixed or heterogeneous pool can then be analyzed by a technique capable of detecting sequence differences (Para 0037).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method that Uhlmann and Nyren by using an amplification primer that does not contain CpGs as suggested by Laird. The method of Laird is advantageous because primers that lack CpG dinucleotides can be used to amplify the sequence between the two primers, regardless of the DNA methylation status of that sequence in the original genomic DNA (Para 0016).

Conclusion

13. No Claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amanda M. Shaw whose telephone number is (571) 272-8668. The examiner can normally be reached on Mon-Fri 7:30 TO 4:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached at 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Amanda M. Shaw
Examiner
Art Unit 1634

BJ FORMAN, PH.D.
PRIMARY EXAMINER